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W. A. Hagan

Tenth International Veterinary Congress, London, 1914.

Johne's Disease (Enteritis chronica paratuberculosa bovis).

REPORT by Mr. OLUF BANG, Copenhagen.

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Johne's Disease (Enteritis chronica paratuberculosa bovis).

REPORT by Mr. OLUF BANG, Copenhagen.

I AM not going to relate in particular the history, etiology and pathological anatomy of the disease[1] referring the reader to the reports from The Hague Congress made by B. Bang, Bongert, Liénaux, Markus, Miessner, and Stuurmann, further to the works of M'Fadyean, K. F. Meyer, and Moussu, and to a monograph on Johne's disease by Twort and Ingram which also contains an extensive bibliography. What I want to emphasize is that to-day nearly all writers agree that it is an infectious specific disease, experimentally transmissible. This was first shown by B. Bang. It is a very chronic disease, and months, nay, even years, may pass between the time of infection and the breaking out of the disease. In many cases, in fact, the disease, if not spreading, will never show any symptoms (own experiences). The cause of the disease is a small acid-fast bacillus living in the intestinal mucous membrane and the mesenteric glands. The intestinal mucous membrane becomes thickened. This thickening is generally specially pronounced in the back part of the small intestine. The mesenteric glands are generally somewhat swollen and succulent. Ulcerations in the bowel or caseous processes in the bowel or the mesenteric glands are never found in Johne's disease as they are in tuberculosis. The symptoms of the disease are intermittent diarrhœa and emaciation. If once clinic symptoms have appeared in the animal it will nearly always perish from the disease sooner or later. After calving the disease often gets worse. No specific treatment is known. Besides, in bovines the disease is also found in other ruminants, thus in the sheep (Vukovic, Stewart Stockman, M'Fadyean, and his collaborators Sheather and Edwards, Twort and Ingram), in deer (M'Fadyean).

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[1] Like K. F. Meyer, I propose to call the disease paratuberculosis, its bacillus the paratubercle bacillus (paratuberculosis), and the tuberculin prepared from it paratuberculin.

O. Bang has observed a typical case in a young goat which lived in the stable of the experimental laboratory in which there were several cows that were sorely stricken by Johne's disease. Moreover Johne's disease has repeatedly been experimentally transmitted to sheep and goats (Twort and Ingram, M'Fadyean). The disease has further been experimentally transmitted to the rabbit by C. C. Twort by intravenous inoculation. C. W. Andersen and O. Bang made a similar observation. It was in the case of a rabbit which on July 13, 1912, had been inoculated subcutaneously with material from the above-mentioned goat on the occasion of some experiments with complement fixing antibodies. The rabbit was killed on January 13, 1914, when Andersen, to his great surprise, found the intestine very much thickened and the intestinal mucous membrane swarming with acid-fast bacilli, which behaved like Twort's bacillus (specific bacillus of enteritis). The rabbit had not shown the slightest symptoms and was in excellent condition. No trace of tuberculosis was found.

Further, a case in a horse has been described by Liénaux. The horse had signs of tuberculosis in two mesenteric glands. The intestinal mucous membrane was very much thickened. Contrarily to what is the case in cattle, a number of small ulcerations appeared in the intestinal mucous membrane.

Since The Hague Congress Twort and Ingram have succeeded in recovering in pure culture the micro-organism that is the cause of Johne's disease. The first notice about it was published in 1910. The success of their cultural experiments was due to Twort's happy thought of adding killed tubercle bacilli to the medium. Twort used Dorset's egg medium. Continued researches showed that the tubercle bacillus might be replaced by different non-pathogenic acid-fast bacilli, among others the *B. phlei*, or extracts of the same. Twort and Ingram have succeeded in producing the disease in calves, sheep, and goats by feeding and intravenous inoculation with the micro-organism they have cultivated, thus confirming in a decisive manner that the disease is a specific disease caused by the acid-fast bacillus found.

Inoculations of guinea-pigs, rabbits and fowls with pure cultures have shown—in conformity with the results of earlier inoculations with material got from animals attacked with enteritis but tuberculosis-free—that the disease is not a form of tuberculosis.

These cultural experiments have been confirmed by researches made by Holth and by M'Fadyean and his collaborators. The present writer, too, has, in several cases, isolated cultures in the way indicated by Twort. Holth and M'Fadyean have shown that Dorset's egg medium might be replaced by serum and serum-agar when, as is the case with the egg medium, acid-fast bacilli or extracts of these are added.

Besides having well deserved of science by their cultivation of the paratubercle bacillus and their indication of a cultural method by means of which anybody can obtain pure cultures of this bacillus, Twort and Ingram have done a work of great practical value that has made it possible (or anyhow will no doubt make it possible) to produce a diagnostic vaccine (paratuberculin) which will no doubt be of great importance in fighting Johne's disease.[2] That it should be possible to produce, by means of the paratubercle bacillus, a paratuberculin that would have the same effect on Johne's disease as tuberculin has on tubercular animals was all the more likely as the present writer, as early as in 1908—thus two years before the pure culture of the paratubercle bacillus was obtained—succeeded in producing a diagnostic vaccine that proved useful against Johne's disease, from another acid-fast bacillus, namely, the avian tubercle bacillus. Twort and Ingram have also, from the outset, directed their attention to this side of the question, taking a great deal of trouble in order to produce a paratuberculin. They have, however, met with great difficulties, the paratubercle bacillus proving very restive to give an abundant growth on the bouillon cultures (even if extracts of acid-fast bacilli were added), which, however, is necessary when a sufficiently active tuberculin is to be produced. As far as the present writer is concerned, who has made many experiments to get the paratubercle bacillus accustomed to grow on the surface,[3] these difficulties were so great that I have provisionally given up the solution of the problem on these lines. Twort and Ingram have evidently met with the same difficulties, for after some experiments of producing a paratuberculin in the same way as used in the case of tuberculin, *i.e.*, heating of the cultures and filtration of the bacilli, they have passed on to making vaccines containing the bacilli, killed by heat, suspended in the bouillon culture. I cannot help thinking that some objections exist to this solution of the problem. Thus healthy animals that do not react to the injection will no doubt sooner or later after the inoculation react when retested, because of their inoculation with the paratubercle bacillus on the first injection; further, in case of doubtful reaction, retesting will for the same reason be impossible till after a long lapse of time. The inoculation will also make impossible the use of other diagnostic methods, such as complement fixing, a method that, in case of strongly affected animals, will most likely prove to be of greater practical value than any vaccine.

Twort and Ingram have tested their vaccine in three animals spontaneously affected and in five calves experimentally infected by intravenous injection. As for the two calves, the temperature was, by mistake, not taken long enough, so it is not possible to judge the result. The other animals reacted.

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[2] The purely technical side of the production of this diagnostic vaccine is, however, in the present writer's opinion, not quite satisfactory as yet.

[3] I have only succeeded in obtaining surface cultures in the form of isolated islets of the size of up to a penny, and this only in a few flasks.

Besides the above-stated objections to the use of diagnostic vaccines containing bacilli, it seems to me that this method cannot be recommended until it has been proved by experiments that healthy bovines never get any rise of temperature consequent on intravenous inoculation of up to 10 c.c. of paratubercle bacillus emulsion.

In their continued researches, Twort and Ingram have also passed on to using subcutaneous inoculation. With the same vaccine as used for the tests with intravenous vaccine they inoculated two experimentally infected goats, both of which reacted in a typical way and also afterwards turned out to be affected. They further made inoculation tests with a new vaccine from a bouillon culture of paratuberculosis. No acid-fast bacilli or extracts from such were added to the bouillon, Twort and Ingram having succeeded in acclimatizing one of their strains to ordinary glycerine-peptone broth. "The cultures were shaken and the large masses of growth allowed to settle. The fluid containing the smaller particles was heated to 65° C. for one hour in a water-bath, and was used without previous filtration." This vaccine was tested on four cows that had contracted the disease spontaneously. Out of these four cows three reacted typically, whereas the fourth failed to react. On *post-mortem* examination they all four proved to be affected by paratuberculosis. The cow that failed to react was particularly sorely stricken. Twort and Ingram think—and no doubt rightly so—that this was the cause of its failing to react. Besides these four cows, twenty-one others were inoculated. Of these ten reacted. Unfortunately no *post-mortem* examination of the animals is published. It is stated, however, that four out of the ten showed clinical symptoms. Whether all those that failed to react were really healthy it is, of course, impossible to form any opinion about. Most of them belonged to a herd among which there were two with clinical symptoms.

Finally, Twort and Ingram have tested five sheep experimentally infected. Three of them reacted typically, two were doubtful according to Twort and Ingram's judgment,[4] which, certainly in my opinion, is rather severe, the sheep with a doubtful reaction showing a rise of temperature to 105° and 105·2° F. (about 40·6° and 40·7° C.) respectively. The three typically reacting sheep were killed and found to be affected.

From these experiments Twort and Ingram conclude that their vaccine " is as reliable a reagent for Johne's disease as tuberculin is for tuberculosis." We hope that this will turn out to be the case. The number of tests with Twort and Ingram's vaccine, however, is, for the present moment, not so large that it can claim to have been fully tested.

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[4] Unfortunately all Twort and Ingram's indications of temperature suffer from the not unessential defect that the temperature before the inoculation is never indicated.

Twort and Ingram call attention to the fact that the maxima of temperature after the inoculation of their vaccine are reached between the fourth and the tenth hour after the injection, thus earlier than in the tuberculin test.

Twort and Ingram have given a great deal of time to the question of using avian tuberculin in Johne's disease, with the result that this means is quite worthless. Thus they write: " As in most biological tests, specificity can only be obtained by an exact system of dosage; and it is for this reason that the use of avian tuberculin in Johne's disease has been proved to be practically worthless. We have seen that in Johne's disease undoubted reactions were obtained by Bang, Miessner, &c., by the use of avian tuberculin, but the reactions were more often negative than positive; and there is no doubt that to attain a larger percentage of positive results the dose of this vaccine would have to be such that it would be beyond the limit of reliability and tubercular or even sound animals might give a reaction."

The present writer is quite ready to concede that avian tuberculin is no ideal means; and I shall be the first to replace it by paratuberculin as soon as I see my way to produce the latter sufficiently efficient (without contents of paratubercle bacilli) and in sufficient quantity, but I honestly think that Twort and Ingram's judgment of the use of avian tuberculin is rather unjust.

Twort and Ingram themselves seem only to have injected avian tuberculin into two animals suffering from Johne's disease. None of the animals reacted. One was an experimental calf, the other a bull strongly affected with Johne's disease. Both animals, moreover, were suffering from tuberculosis.

On June 27 the calf was inoculated with a paratuberculin also containing tuberculin, killed tubercle bacilli having been added to the broth. The reaction was positive. On July 27 it was inoculated with tuberculin and reacted (having tuberculosis). On August 28 it was inoculated with 8 c.c. of paratuberculin without reacting. Finally, nine days later, on September 6, it was inoculated with avian tuberculin without reacting. Twort and Ingram do not seem to have been aware that their way of proceeding should rather be called a bringing-on immunity than a comparison between different vaccines. The bull was strongly affected with Johne's disease; on the *post-mortem* examination it turned out to be also suffering from tuberculosis. It did not react on the first test to the mixture of paratuberculin and tuberculin, as did the experimental calf. This result did not seem *in se* to qualify it to serve as control on further tuberculin tests. Unfortunately, Twort and Ingram do not state any temperatures at all for this experimental series, which would have been a highly desirable thing, these authors being, as it appears, rather exacting in respect of the height of rise of temperature they want as a mark of positive reaction. Thus in speaking of some experiments made by Male with avian tuberculin these authors object to a rise of temperature of 104° F. (40° C.), which, in my opinion, is unjustifiable. According to my rather great experience, a rise of temperature reaching 39·8° C. (103·6° F.) in full-grown animals may unhesitatingly be taken as positive, if the temperature before the inoculation is 39° C. (102·2 F.) or below. The present writer cannot find any difficulty in such an appreciation. A real drawback with respect to avian tuberculin is, on the contrary, the fact that a much lower rise of temperature may indicate reaction and that animals strongly affected may fail to react altogether. But the same thing may occur in cases of tuberculin tests of tubercular animals, although less frequently, certainly, than on avian tests of animals affected with paratuberculosis. Also Twort's experiments with paratuberculin show that with a tuberculin made from the specific micro-organism this difficulty cannot be turned altogether either.

In the same way it is only a hypercritical judgment of the reactions that makes it possible for Twort and Ingram to arrive at the conclusion that in Johne's disease the avian tuberculin tests have more frequently shown negative than positive results.

The following communications with regard to the use of avian tuberculin as diagnostic means in Johne's disease have as yet been published.

Male's tests: Nine animals that reacted, one with a doubtful reaction. Of these four that reacted were killed; in three cases paratuberculosis was manifest. As to the fourth, Stockman states that although he did not find any paratuberculosis he still thinks that the animal was affected with Johne's disease. Malm's tests comprehending two calves experimentally infected that both reacted on the first test, and, being killed, proved to be affected. The calves did not react on all following tests, especially so when on a following test they were injected with a smaller dose than the one used the first time. This is a very common feature with avian tuberculin tests and is due, no doubt, to the relative facility with which the animals become immune. Miessner's tests comprehending five experimentally infected calves, of which four reacted; further, some goats and dogs, of which one goat only showed a marked rise of temperature. No communication concerning the *post-mortem* examinations have as yet been published. Further, the numerous tests made by the present writer, the results of which certainly were not that most of the infected animals did not react. Finally, tests made by M'Fadyean, Sheather, and Edwards. They inoculated fourteen cows, of which thirteen had marked clinical symptoms, while the fourteenth showed no symptoms but "was picked out from an apparently healthy herd" on account of the reaction. On being killed they all proved to be affected. Below the temperature before and the maximum temperature after the inoculation are given :—

	Before inoculation.	After inoculation.	
I.	38·8° C. (101·8° F.)	39·7° C. (103·4° F.)	R.
III.	38·7° C. (101·6° F.)	39·3° C. (102·8° F.)	
	38·6° C. (101·4° F.)	40·2° C. (104·4° F.)	R.
IV.	38·4° C. (101·2° F.)	39·3° C. (102·7° F.)	R. ?
V.	37·8° C. (100° F.)	39·1° C. (102·4° F.)	R. ?
VI.	38·9° C. (102° F.)	39·8° C. (103·6° F.)	R.
VII.	38·3° C. (101° F.)	39° C. (102·2° F.)	O.
VIII.	38·4° C. (101·2° F.)	39·9° C. (103·8° F.)	R.
	38·9° C. (102° F.)	41° C. (105·8° F.)	R.
XI.	38·4° C. (101·2° F.)	40° C. (104° F.)	R.
XII.	38·4° C. (101·2° F.)	39·4° C. (103° F.)	R.
XIII.	38·7° C. (101·6° F.)	39·4° C. (103° F.)	R. ?
XIV.	38·7° C. (101·6° F.)	39·6° C. (103·2° F.)	R.
XVI.	38·7° C. (101·6° F.)	41·3° C. (106·4° F.)	R.
	38·8° C. (101·8° F.)	39·4° C. (103° F.)	
XVII.	38·8° C. (101·8° F.)	39° C. (102·2° F.)	O.
XVIII.	38·6° C. (101·4° F.)	38·9° C. (102·1° F.)	O.

According to my judgment, I should say that out of fourteen animals eight had reacted, three reacted doubtfully, three did not react.

Case III did not react on the first test, which no doubt was due to the fact that the taking of temperature ceased fifteen hours after inoculation. The animal had not till on this test reached 39·3° C. as the maximum temperature. I have seen several times that the reaction does not manifest itself till eighteen to twenty hours after inoculation.

Considering that the animals in question showed marked clinical symptoms, great emaciation and, for the greater number of animals, intense diarrhœa, it seems to me that the result cannot be called a bad one. The tuberculin was applied in a dose of 8 c.c., whereas I generally apply it in a dose about double that quantity (1·5 c.c. to 2 c.c.) concentrated tuberculin and 8 c.c. of a solution of carbolic acid, which, I think, is preferable, especially in the case of animals with clinical symptoms as these, as already stated, often give a slight reaction or no reaction at all.

Twort and Ingram, writing about the results of these tests, say: "We consider that the results obtained by these workers afford further proof that the avian tuberculin test is not a reliable means of diagnosing cases of Johne's disease, for in spite of the large doses of vaccine that were given, the majority of the cases failed to react, a result which is in agreement with that obtained by other investigators." It is, of course, quite true that avian tuberculin is no absolutely reliable diagnostic means—the specific vaccine as produced by Twort and Ingram is not, unfortunately, so either—whether there was no reaction in the majority of cases is a question that depends on judgment. But the judgment appears to me rather severe when Cases VI and VIII that showed a rise of temperatures from 38·9° to 39·8° C. (103·6° F.), and from 38·4° to 39·9° C. (103·8° F.) respectively are marked as ÷ (?). I should add, however, that Twort and Ingram, too, are not quite unwilling to concede that a rise of temperature to 103·6° F. may, at least under certain conditions, be marked as a positive reaction. Thus on their tests with their own vaccine they mark 103·6° F. (39·8° C.), as positive and as a fair reaction (Case XV) stating, by way of excuse for this appreciation, that the tested animals were "in a bleak, open position, and all were tested on a cold day." There is not much else to be said to this but that just as it is unquestionable that exterior conditions may influence the rise of temperature, so it is impossible to prove that these, in the present case, were the real cause why the temperature did not rise sufficiently high. I am at a loss to understand why Twort and Ingram find 8 c.c. "a large dose," using as they do their own vaccine in a quantity of from 5 c.c. to 10 c.c.

Later K. F. Meyer has tested four experimentally infected calves with avian tuberculin. Three of these reacted, the fourth did not, though all four on the *post-mortem* examination proved affected. One experimentally infected lamb reacted, one goat did not. Some spontaneously infected cows, all of which were severely affected by the disease, failed to react. Twort and Ingram seem to fear that the dose of from 1·5 c. to 2 c.c. of concentrated avian tuberculin, as used by me, should cause even normal animals to react. This fear is, according to my experience, quite without foundation. But, on the other hand, it is an indisputable fact that a certain percentage of tubercular animals will react to the avian tuberculin test; a fact I am going to speak about later.

As to my own researches, I have mainly endeavoured to eradicate the paratuberculosis in stocks by isolating reacting animals (Bang's system of fighting tuberculosis), but without success as yet. The cause, most likely, was that a few animals not showing any clinical symptoms either quite failed to react or else only reacted slightly and consequently remained among the healthy stock infecting it little by little. That this really was the cause could be clearly demonstrated in several places.

In all about 5,600 animals belonging to fifty odd stocks have been examined (on first tests) in Denmark. Several of these stocks have been examined more than once. Some of the stocks were rather large, between three and four hundred head. With a few exceptions nearly all the stocks were entirely tuberculosis-free. Of the bovines about 4,400 were Jersey cattle, about 1,200 red Danish breeds. Between 5 and 40 per cent. of the animals in the different stocks reacted. *There was always a much larger number of the grown cattle than of the young ones that reacted;* the latter in many places being quite healthy. The cause of this is no doubt that on the larger farms the young cattle are not taken into the stables until they are going to calve.

I give below some figures clearly illustrating the above fact. All the figures are from first tests of the stocks.

B. and W. ...	Of 139 head more than 2 years old, 63 = 45·3 per cent reacted
	Of 31 head under 2 years old, none reacted.
S . gaard ...	Of 69 head more than 2 years old, 26 = 37·7 per cent. reacted
	Of 25 head under 2 years old, none reacted.
L . . hoj ...	Of 67 head more than 2 years old, 23 = 34·5 per cent. reacted.
	Of 26 head under 2 years old, one reacted.
N gaard ...	Of 148 head more than 2 years old, 16 = 10·8 per cent. reacted
	Of 73 head under 2 years old, 4 = 5·5 per cent. reacted.
Lr . . . gaard ...	Of 99 head more than 2 years old, 36 = 36 per cent. reacted.
	Of 70 head under 2 years old, 7 = 10 per cent. reacted.
S . . . p ...	Of 291 cows, 37 = 13 per cent. reacted.
	Of 77 young cattle, 6 = 8 per cent. reacted.
S . . . n ...	Of 85 more than two years old, 19 = 23·5 per cent. reacted.
	Of 29 under 2 years old, none reacted.
Ld . . . gaard ...	Of 89 more than 2 years old, 8 = 9 per cent. reacted.
	Of 19 under 2 years old, none reacted.

All these stocks, to which a good many others could easily be added, all showing similar results, are tuberculosis-free and always have been so. They are all Jersey stocks founded on direct imports from Jersey, where no tuberculosis exists. Thus it is very easy to keep them free of tuberculosis provided no strange cattle are introduced into the farm. They are tested every year by means of tuberculin.

As to fighting Johne's disease through isolation of the reacting animals the difficulty is in the fact that a few animals, really affected but apparently healthy, fail to react It is of no importance in this connection that animals with marked clinical symptoms (intense emaciation and diarrhœa) fail to react, as nobody would think of placing such with a healthy herd.

It is fully in conformity with the above-stated experience that the percentage of reaction in several stocks will fall on the first tests to rise again when the remaining infectious animals that have "cheated" on the test have infected a sufficient number of the healthy ones. A few examples will show this :—

Gr . . . gaarden : The stock was here severely affected with paratuberculosis. Thus in the year 1908-1909 of about 150 animals 22 had been removed because of Johne's disease. The stock was tested for the first time in March, 1909.

March, 1909.—Of 122 head 42 = 34·4 per cent. reacted. (The reacting animals were isolated in a special stable on the farm.)

November, 1909.—Of 69 head, 9 = 13 per cent. reacted. (All reacting animals of the farm were sent to a neighbouring farm.)

April, 1910.—Of 93 head, none reacted.

October, 1910.—Of 93 head, 3 = 3·2 per cent. reacted.

April, 1911.—Of 113 head, 8 = 7 per cent. reacted.

April, 1912.—Of 120 head, 8 =6.7 per cent. reacted.

Thus the result cannot be said to be satisfactory.

Though all reacting animals were at once removed from the farm a not inconsiderable number of animals failed to pass on later tests. It was not possible to point out directly the source of infection as, to my knowledge, no cases of clinically demonstrable paratuberculosis appeared among the healthy section.

K . . .p. Heavy losses had been suffered in the stock owing to Johne's disease. The whole stock was tested with avain tuberculin.

May, 1910.—Of 280 head, 78 = 27·9 per cent. reacted.

October, 1910.—Of 204 head, 21 = 10·3 per cent. reacted.

April, 1911.—Of 211 head, 12 = 5·7 per cent. reacted.

November, 1911.—Of 200 head, 9 = 4·5 per cent. reacted.

One cow that had passed all four tests was killed, as it was wasting and gave too little milk. In the preparation of intestine sent in paratubercle bacilli were demonstrated in abundance.

October, 1912.—Of 220 head, 20 = 10 per cent. reacted.

One cow of the healthy section was slaughtered. On examining the intestine sent in paratubercle bacilli were demonstrated.

After each test all reacting animals had been sent off to another farm so that the separation between the reacting and non-reacting animals was ideal. No wonder that the result was no better considering that capital sources of infection were demonstrable within the stock itself. During the first eighteen months no case of paratuberculosis among the healthy section had occurred.

L . .gaard.—The stock suffered very heavy losses from paratuberculosis.

August, 1909.—Of 169 head, 42 = 24·9 per cent. reacted; of grown animals 36 per cent. reacted.

The separation of the reacting from the non-reacting animals was, as far as I know, not made till several months after injection.

In February, 1910, the assurance society took over in a lot fourteen of the reacting animals. They all showed marked symptoms of the disease. The cows were slaughtered. On examination of the sections of bowels sent in paratuberculosis were demonstrated in all cases.

The following reactions were shown by the animals on the tests in August, 1909:—

Temperatures before inoculation at 8 p.m.	9 hours later	11 hours later	13½ hours later	16 hours later	18 hours later	20 hours later
C.	C.	C.	C.	C.	C.	C.
38·5°	38·7°	39·5°	39·4°	39·6°	38·7°	38·5°
38·6°	38·7°	39·9°	40·3°	39·9°	39·2°	38·6°
38·7°	39·2°	39·8°	39·4°	39·5°	38·7°	38·4°
39°	40·4°	40·5°	40·6°	39·9°	39·2°	39°
38·9°	40·2°	39·1°	38·6°	38·9°	39·1°	39·2°
38·4°	40·2°	39·3°	38·7°	39°	39°	39·6°
38·7°	40·1°	40·7°	39·4°	39·5°	39·8°	39·4°
39·3°	39·4°	40°	40°	40·2°	39·7°	39·3°
39°	39·5°	40°	40·4°	40·3°	39·4°	39·5°
38·9°	39·2°	39·3°	40°	40°	39·1°	38·7°
38·6°	38·7°	39·2°	40°	39·9°	39°	38·8°
38·9°	38·6°	38·8°	38·9°	40·1°	39·5°	39°
39°	40·1°	40·5°	40°	40·1°	40·1°	39·6°
39°	40·6°	39·3°	39·7°	40·5°	40·8°	39·6°

For the inoculation 2 c.c. of concentrated avian tuberculin + 8 c.c. of solution of ½ per cent. of carbolic acid were used. The stock was retested.

May, 1910. Of 118 healthy animals 32 = 27 per cent. reacted.

One cow of the healthy section was removed one month after the injection owing to paratuberculosis (the case was not microscopically controlled).

Of 25 reacting animals 19[5] = 76 per cent. reacted on being retested.

From the reacting section 9 animals were removed on account of paratuberculosis.

May, 1911. Of 97 healthy animals 16 = 16·5 per cent. reacted.

1912. The owner stated that two cases of paratuberculosis had occurred in the healthy section. In the reacting section 10 animals were removed owing to paratuberculosis.

The reacting section was separated on the farm itself.

The stock was not tested later.

The following will show the state of a Jersey stock in which there is hardly any loss from paratuberculosis on applying the avian tuberculin test:—

E . . . gaard.—On the test reacted:—

July, 1909.—Of 70 head, 5 = 7 per cent.

May, 1910.—Of 69 healthy head none reacted.

Of five previously reacting three reacted again. These three were killed. On microscopic examination of the bowels sent they turned out to be affected (paratuberculosis demonstrated).

February, 1911.—Of 70 healthy ones, 3 = 4 per cent. reacted.

March, 1912.—Of 99 healthy ones, 3 = 3 per cent. reacted.

April, 1913.—Of 95 healthy ones one showed a doubtful reaction.

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[5] Two cows that reacted on the preceding test but not on this were now greatly emaciated and had intense diarrhœa. They were killed shortly after. Numerous paratubercle bacilli were demonstrated in sections of bowels sent in.

In a few small stocks the result was that a single animal only reacted. This being sold no other animal reacted on a retest. On the other hand, it has not yet been possible, as already stated, to crush the disease in any severely infected large stock. The conditions, however, in most of these have been greatly improved. This is, no doubt, greatly due to the fact that, since the disease has become known, no severely affected animal is left for any length of time with the stock.

In order to try whether there was any risk of getting reactions on the avian tuberculin test in stocks in which the disease was unknown eighty-seven tuberculosis-free red cows were injected. Of these one reacted. Some months previously, however, sixty-nine Jersey cross-breeds had been removed to the same stable, two of which reacted, and nineteen head pure Jerseys, of which five reacted. The reacting red cow was standing next to a Jersey bull that reacted to the test.

For the test 2 c.c. of concentrated avian tuberculin + 8 c.c. of a solution of ½ per cent. of carbolic acid was used. It is evident from this that a dose of 2 c.c. of concentrated avian tuberculin fails to produce a reaction in healthy cows.

It would be a thing of the greatest interest to inquire into the fates of the reacting cows; whether they all perish from the disease or whether part of them live on without ever showing any signs of the disease and going on giving a full yield. In order to examine this problem the present writer has during five years had sent to him intestinal pieces and mesenteric glands of almost all the cows that were removed from a large tuberculosis-free Jersey stock, tested with avian tuberculin for the first time in April, 1909. It has thus been possible to follow the fates of the reacting cows.

Of fifty-four cows reacting to the test of April, 1909, eleven are still living on, hale and hearty, without ever showing any morbid symptoms. Forty-three were killed or died. For more than half of them there is good evidence to show that they have shown no morbid symptoms (emaciation or diarrhœa) but were removed for other reasons (old age, sterility, too little yield, &c.). In thirty-seven of forty-three, that is in 86 per cent., of the specimens of intestine and mesenteric glands sent in a microscopic examination showed the presence of paratubercle bacilli, a most satisfactory result, especially when considering that only small intestinal pieces and a few mesenteric glands were examined. In doubtful cases sections of intestine and mesenteric glands were made, and the results obtained through this examination decided the judgment. One of the five cows (338) in which the microscopic examination failed to demonstrate the presence of paratubercle bacilli had repeatedly been suffering from intermittent diarrhœa and was removed because of the marked clinical symptoms (emaciation and intense diarrhœa) it showed. Such cases in which, in spite of clinical symptoms, paratubercle bacilli are not demonstrable at all or not demonstrable without a very close examination have been met with by most of those that have been working with paratuberculosis. Below I am going to give the reactions that appeared in the above-mentioned fifty-four cows. The first year they were injected twice a year with avian tuberculin.

In July, 1912, and January, 1913, some of the cows were submitted to the "intradermic test" and the complement-fixation test. Of the figures placed in the face of "intradermic test" the first indicates thickness of the skin before injection with avian tuberculin $\frac{1}{10}$ c.c. of concentrated avian tuberculin used, the second, third and fourth figures thickness of skin forty-eight, seventy-two, and ninety-six hours after inoculation respectively. In several cases measurements were only made forty-eight and seventy-two hours after inoculation. A difference in the thickness of the skin of 4 mm. before and after inoculation is taken as positive reaction.

		Temperatures before inoculation at 8 p m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 47 ...	6.4.09 ...	37·9°	38·5°	39·3°	39·2°	39·5°	39·3°	38·9°	
	4.12.09 ...	38·2°	38·6°	38·8°	39°	39·5°	40·1°	40°	
	12.4.10 ...	38·4°	39·3°	38·8°	39·1°	38·5°	38·8°	39·9°	39·4°
	21.11.10 ...	38·4°	38·9°	39·3°	39·3°	38·1°	39·5°	39·4°	39·3°

Slaughtered January, 1911. Paratuberculosis in smears from the bowels.

		Temperatures before inoculation at 8 p m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 103 ...	6.4.09 ...	38·2°	38·4°	38·8°	37·8°	38·4°	39·4°	39·1°	
	4.12.09 ...	38·4°	39°	39°	39·3°	39·1°	39·6°	39·8°	
	12.4.10 ...	38·4°	38·9°	39·4°	39·1°	38·8°	39·4°	40·1°	39·4°

Slaughtered November, 1910. Paratuberculosis in smears from the bowels.

		Temperatures before inoculation at 8 p m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 128 ...	6.4.09 ...	38·4°	38·7°	38·5°	38·1°	39°	40°	39·8°	
	4.12.09 ...	38°	38·9°	38·8°	38·4°	38·3°	38·8°	39·4°	39·2°
	12.4.10 ...	38°	38·5°	39·3°	39·4°	39·1°	39·5°	39°	38·4°
	21.11.10 ...	38·3°	39°	39·1°	37·5°	38·8°	38·5°	38·6°	
	31.3.11 ...	38·1°	38·5°	38·7°	38·2°	38·4°	39°	38·8°	38·4°
	21.11.11 ...	38·1°	38·4°	38·9°	38·9°	38·5°	38·6°	38·4°	
	16.4.12 ...	38·5°	38·3°	38·8°	38·6°	38·6°	38·8°	38·3°	

Is still living.

		Temperatures before inoculation at 8 p m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.
No. 163 ...	6.4.09 ...	38·5°	39·6°	40·5°	40·7°	40·2°	39·8°	39·5°

Slaughtered August, 1909. Paratuberculosis in smears from the bowels.

		Temperatures before inoculation at 8 p m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 177 ...	6.4.09 ...	38·1°	38·9°	39°	38·5°	39·5°	39°	38·8°	
	4.12.09 ...	38·7°	38·8°	39·3°	39·2°	38·7°	39·7°	39·8°	40·2°
	12.4.10 ...	38·6°	38·5°	38·8°	39·2°	38·1°	39°	39·1°	38·6°
	21.11.10 ...	38·7°	38·8°	39·5°	39·2°	38·8°	39·5°	39·5°	39·5°
	31.3.11 ...	38·3°	38·8°	39°	38·7°	38·8°	38·3°	37·8°	38·6°
	21.11.11 ...	38°	38·4°	38·8°	38·2°	39·4°	39·3°		
	16.4.12 ...	38·4°	38·7°	38·9°	38·6°	38·5°	38·6°	38·6°	

Is still living.

		Temperatures before inoculation at 8 p.m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 186	6.4.09	38·3°	39·1°	39·7°	39·2°	39·4°	39·3°	38·9°	
	4.12.09	38·3°	38·4°	38·9°	39·2°	39°	40°	39·2°	
	12.4.10	38·2°	38·7°	39·6°	40·2°	40°	40·2°	39·2°	
	21.11.10	39°	38·7°	39·9°	40·2°	39·9°	39·8°	39·8°	
	31.3.11	38·3°	38·7°	39·4°	39°	39·2°	39·5°	39·2°	39·6°
	21.11.11	38·1°	38·4°	38·9°	38·9°	38·5°	38·6°	38·4°	
	16.4.12	38·6°	38·2°	38°	38·2°	38·3°	38·6°	38·5°	
	10.7.12	Intradermic test : 6, 10, 10, 12.							

Reacted on complement-fixation test. Slaughtered July 21, 1912. One paratuberculosis in smears from the bowels; in sections giant cells with bacilli.

No. 190	6.4.09	38·3°	39·4°	40·1°	39·4°	40·6°	39·3°	39·5°	
	4.12.09	39·4°	39·4°	39·9°	40°	39·8°	40·2°	40·4°	
	12.4.10	38·7°	39·5°	39°	39°	38·8°	39·2°	39·2°	38·9°

Slaughtered May, 1910. Paratuberculosis in smears from the bowels.

No. 202	6.4.09	38·1°	38·4°	39·2°	40·2°	41·1°	40·2°	39·4°	
	4.12.09	38·2°	38·2°	38·9°	38·8°	38·8°	39·5°	40·1°	
	12.4.10	38·4°	38·8°	39·3°	39·6°	39·8°	40·7°	40·3°	39·5°
	21.11.10	38·3°	38·7°	39·1°	39·7°	38·7°	38·5°	38·8°	
	31.3.11	38·3°	38·5°	38·5°	38·5°	38·8°	39°	39·1°	38·7°

Slaughtered April, 1911. Paratuberculosis in smears from the bowels; has shown no signs of paratuberculosis. Slaughtered because it has ceased to react.

No. 207	6.4.09	38·7°	40°	40·6°	40·2°	40·3°	40·6°	40·5°

Slaughtered July, 1909. Paratuberculosis.

No. 220	6.4.09	38·2°	39°	39·2°	39·1°	39·9°	41·1°	39·3°	
	4.12.09	38·2°	38·8°	39·3°	39·2°	38·3°	38·7°	38·5°	
	12.4.10	38·6°	39·1°	39°	38·8°	38·2°	38·4°	38·7°	38·5°
	21.11.10	38·5°	38·8°	39·2°	39·1°	38·5°	38·6°	38·5°	
	31.3.11	38·3°	38·6°	39°	38·3°	38·8°	38·6°	38·7°	38·4°
	21.11.11	38·2°	38·7°	38·8°	38·9°	38°	38°	38·3°	

Slaughtered May, 1912. Paratuberculosis.

No. 234	6.4.09	38·6°	39·2°	39°	37·5°	38·7°	39·7°	39·9°	
	4.12.09	38·5°	38·4°	39·6°	39°	40·1°	39·2°	39°	
	12.4.10	38·6°	39°	39·5°	39·4°	39°	39·2°	39·3°	39·5°
	21.11.10	38·3°	38·7°	39°	38·9°	38·5°	39°	39°	38·9°
	31.3.11	38·7°	39°	39°	38·6°	38·5°	39·2°	38·9°	38·9°
	21.11.11	38·3°	38·3°	38·8°	38·5°	38·9°	38·3°	38·1°	
	16.4.12.	38·4°	38·5°	38·7°	38·1°	38·3°	37·9°	37·8°	

Slaughtered July, 1912. No Paratubercle bacilli in smears from the bowels.

		Temperatures before inoculation at 8 p.m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 239 ...	6.4.09 ...	38·2°	39°	39·4°	39·1°	39°	39.1°	39·2°	
	4.12.09 ...	38.8°	39·2°	40·3°	41°	40·6°	40°	40°	
	12.4.10 ...	38·5°	38·4°	39·3°	39·7°	39·8°	40°	39·5°	39·3°
	21.11.10 ...	38·6°	39·4°	40·5°	40·6°	40·1°	40·3°	39·7°	
	31.3.11 ...	38·4°	39·1°	39·6°	39·3°	39·7°	39·7°	39·4°	38.0°

Slaughtered April, 1911. Paratuberculosis.

No. 246 ...	6.4.09 ...	38·1°	38·6°	39·1°	39·1°	39·5°	39·4°	39·3°	
	4.12.09 ...	38·4°	39°	39·7°	39·5°	39·8°	39·8°	39·6°	
	12.4.10 ...	38·4°	38·3°	39°	39·3°	38·8°	39°	38·5°	39.5°

Slaughtered November, 1910. No Paratubercle bacilli in smears from the bowels.

No. 248 ...	6.4.09 ...	38·5°	39·3°	40°	39·4°	40·1°	40·5°	39·9°
	4.12.09 ...	38·1°	39°	39·1°	39·3°	39·1°	39°	38·7°
	12.4.10 ...	38·3°	39·1°	39·4°	40·1°	39·7°	40·4°	40·6°

Slaughtered November, 1910. Paratuberculosis in smears from the bowels.

No. 249 ...	6.4.09 ...	38·3°	39·4°	40·3°	39·6°	39·5°	39·5°	38·8°	
	4.12.09 ...	38·3°	38·8°	39·1°	39·4°	39·6°	40·2°	39·4°	
	12.4.10 ...	38·8°	38·5°	38·9°	38·8°	38·8°	39·4°	38·7°	
	21.11.10 ...	38·9°	38·8°	39·2°	39°	38·9°	39·5°	39·5°	
	31.3.11 ...	38·6°	38·8°	39°	37·7°	38·3°	38·8°	38·8°	38·6°

Died July, 1911, of paratuberculosis. Many paratubercle bacilli.

No. 259 ...	6.4.0.9 ...	38·4°	39·2°	39·6°	38·8°	39·9°	39·7°	39·1°	
	4.12.09 ...	38·4°	39·3°	39·6°	39·2°	38·6°	39°	39·1°	
	12.4.10 ...	38·6°	39·1°	39·6°	39·8°	39°	40°	40·3°	39·3°

Slaughtered September, 1910. Many paratubercle bacilli.

No. 268 ...	6.4.09 ...	38·2°	39·3°	40·4°	39·8°	39·9°	39·8°	38·8°	
	4.12.09 ...	38°	38·7°	38·8°	38·9°	39·1°	39·5°	39·8°	
	12.4.10 ...	38·1°	40·1°	40·6°	40·5°	40·5°	40·5°	40·6°	39·5°

Died of paratuberculosis, September, 1910. Few bacilli.

No. 269 ...	6.4.09 ...	38·2°	38·9°	39·3°	39·7°	40·3°	39·8°	39·4°	
	4.12.09 ...	38·1°	38·7°	39·8°	39·7°	40°	39·9°	39·8°	
	12.4.10 ...	38·3°	38·8°	39·4°	39·3°	39·3°	39·8°	39·2°	38·9°

Slaughtered November, 1910. Paratuberculosis. Slaughtered because not pregnant.

No. 276 ...	6.4.09 ...	38·2°	39·9°	40·2°	39·3°	39·8°	39·9°	39·6°	
	4.12.09 ...	38·8°	39·1°	39°	39·2°	39·6°	39·9°	39·7°	
	12.4.10 ...	38·3°	38·8°	39·1°	38·8°	38·6°	38·8°	38·2°	38·9°
	21.11.10 ...	38·6°	39°	39·8°	39·5°	39·1°	39·3°	39·6°	39·2°
	31.3.11 ...	38·7°	39·1°	38·9°	37·6°	38·6°	39·5°	39·8°	39°

Slaughtered April, 1911. Many paratubercle bacilli.

		Temperatures before inoculation at 8 p.m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 291	6.4.09	38·4°	39·1°	39·4°	39·2°	40°	40·1°	40·1°	
	4.12.09	39°	38·5°	39·1°	38·8°	38·7°	38·8°	38·8°	
	12.4.10	38·4°	38·8°	38·7°	39°	38°	38·6°	39·2°	38·8°
	21.11.10	38·3°	38·7°	39°	39·2°	38·8°	39·4°	39°	
	31.3.11	38·3°	38·8°	38·9°	38·5°	39·2°	40·2°	40·3°	39·8°
	21.11.11	38°	38·7°	39·2°	39·3°	39·9°	39·1°	38·9°	
	16.4.12	38·5°	39°	37°	38·1°	38·3°	38·6°	38·7°	

January, 1913.—Intradermic test, 5, 9, 9, 11; complement-fixation test negative. Is still living.

No. 292	6.4.09	38·4°	39·7°	40·6°	40·8°	40·5°	40°	39·2°	
	4.12.09	38·9°	38·9°	39·5°	40°	40·7°	40·6°	40·3°	
	12.4.10	38·4°	38·7°	39·2°	39·3°	39·3°	40·5°	40·1°	39·4°
	21.11.10	39·4°	39·7°	40·3°	40·6°	39·9°	40·7°	39·7°	

Died of paratuberculosis, February, 1911. Many paratubercle bacilli.

No. 295	6.4.09	38·2°	39°	39·1°	38·8°	40·3°	40·8°	40·9°	
	4.12.09	39°	39·4°	40·4°	40·6°	40°	40°	39·6°	
	12.4.10	38·9°	39°	40°	40·6°	39·6°	40·2°	39·6°	
	21.11.10	38·4°	39·1°	40·7°	40·8°	39·5°	39·5°	39·4°	
	31.3.11	38·1°	38·9°	39·1°	38·7°	38·7°	39°	39°	38·8°
	21.11.11	39	39·3°	39°	38·8°	39·1°	39·4°	38·4°	
	6.4.12	38·5°	39°	38°	38·5°	38·5°	38·3°	39°	

July, 1912.—Intradermic test, 6, 6, 10, 11; complement-fixation test negative. Slaughtered November, 1913. No paratuberculosis in smears from the bowels.

No. 300	6.4.09	38·1°	39°	39·5°	39·5°	40·1°	39·4°	38·8°	
	4.12.09	38·1°	38·7°	39·2°	39·2°	38·6°	38·6°	38·5°	
	12.4.10	38·2°	38·6°	39·4°	39·3°	38·8°	39·5°	39·1°	38·5°
	21.11.10	38·4°	38·7°	39·1°	38·9°	38·7°	39°	39°	38·9°
	31.3.11	38·1°	38·9°	38·9°	38·3°	38·4°	38·4°	38·4°	
	21.11.11	38°	37·8°	38·8°	38·4°	38·3°	38°	37·9°	
	16.4.12	38·8°	38·7°	38·8°	38·3°	38·7°	38·1°	38·2°	

January, 1913.—Intradermic test, 7, 12, 14, 14; complement-fixation test negative. Is still living.

No. 304	6.4.09	38·3°	38·9°	38·9°	38·1°	39·3°	40·1°	39·1°	
	4.12.09	38·2°	38·7°	39·3°	39°	39·2°	39°	39°	
	12.4.10	38·2°	38·7°	39·1°	39°	38·6°	38·7°	38·7°	38·1°
	21.11.10	39°	38·7°	39·2°	39·3°	39·3°	39·2°	38·9°	
	31.3.11	38·3°	38·7°	38·8°	37·4°	38·7°	38·8°	38·7°	38·5°
	21.11.11	38·6°	38·9°	39·1°	38·9°	38·9°	39·1°	39·2°	
	16.4.12	38·7°	38·5°	38·7°	37·7°	38·6°	38·5°	38·2°	

January, 1913.—Intradermic test, 5, 10, 14, 16; complement-fixation test negative. Slaughtered August 13. No paratubercle bacilli.

		Temperatures before inoculation at 8 p.m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.
No. 307	6.4.09	38·4°	39·7°	40·1°	40·3°	40·3°	40·2°	39·3°
	4.12.09	38°	39·4°	40·3°	40·4°	40°	39·8°	39·3°
	12.4.10	38·7°	39·9°	41°	41·1°	39·7°	39·8°	38·9°

Slaughtered November, 1910; paratuberculosis. Slaughtered because of its giving too little milk.

No. 310	6.4.09	39°	39·9°	40·3°	39·6°	39·6°	39·4°	39·9°

Slaughtered August, 1909; paratuberculosis in smears from the bowels. Slaughtered because of its giving too little milk.

No. 311	6.4.09	38·2°	39·6°	39·7°	39·7°	40·4°	39·2°	38·6°

Slaughtered May, 1910; no paratubercle bacilli. Slaughtered because of its giving too little milk.

No. 322	6.4.09	38·3°	39·4°	39·6°	39·5°	40·2°	39·8°	39·5°

Slaughtered August, 1909. Paratuberculosis in smears from the bowels.

No. 331	6.4.09	38·4°	39·7°	40·7°	39·5°	39·7°	39·5°	38·7°

Slaughtered October, 1909. Many paratubercle bacilli.

No. 336	6.4.09	38·3°	40°	40°	39·2°	39·5°	39·3°	39·5°

Slaughtered October, 1909. Many paratubercle bacilli; had diarrhœa.

No. 337	6.4.09	38·4°	38·9°	39·4°	39·6°	40·8°	40·7°	40°	
	4.12.09	38·4°	38·9°	39·1°	39·1°	38·9°	40°	40·1°	
	12.4.10	38·8°	39°	39·3°	39·6°	39·4°	39·7°	40·8°	39·8°
	21.11.10	38·8°	39°	39·4°	39·2°	38·5°	38·8°	38·6°	38·5°
	31.3.11	38·3°	40°	39·2°	38·5°	38·5°	38·9°	38·5°	38·6°
	21.11.11	38·5°	38·5°	38·8°	38·8°	38·8°	38·6°	38·4°	
	16.4.12	38·7°	38·7°	38°	38·4°	38·5°	38·7°	38·7°.	

July, 1912.—Intradermic test, 5, 9, 9, 9; complement-fixation test negative. Is still living.

No. 338	6.4.09	38·4°	39·2°	40·7°	39·6°	40·6°	40·1°	40·7°	
	4.12.09	38·9°	39·2°	40·2°	40·6°	40·1°	40·3°	40·2°	
	12.4.10	38·5°	38·8°	40·3°	40·9°	40·3°	40°	39·5°	
	21.11.10	39·5°	39·6°	39·8°	40°	39·7°	40·5°	40°	
	31.3.11	38·5°	39·8°	39·8°	39·2°	40·1°	40·2°	40°	40·6°
	21.11.11	39·1°	40°	40·4°	40·3°	40·6°	39·2°	39·3°	
	16.4.12	38·6°	39·1°	38·1°	38·5°	38·9°	38·9°	38·9°	

July, 1912.—Intradermic test, 5, 12, 12, 13; complement-fixation test positive. January, 1913.—Complement-fixation test positive. Slaughtered October, 1913. No paratubercle bacilli; has several times been suffering from periodical diarrhœa.

		Temperatures before inoculation at 8 p.m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 343 ...	6.4.09 ...	37·9°	39·2°	39·8°	39°	40·2°	38·9°	38·8°	
	4.12.09 ...	38°	38·8°	39·9°	40·4°	40·3°	39·5°	39°	
	12.4.10 ...	38·3°	38·7°	39°	39°	38·8°	39·1°	39·3°	38·6°
	21.11.10 ...	38·7°	39·3°	39·5°	39·9°	39·4°	40°	39·4°	
	31.3.11 ...	38·3°	39·4°	39·5°	38·5°	39·3°	39·8°	39·7°	39·1°

Died of paratuberculosis May, 1911; many paratubercle bacilli.

No. 346 ...	6.4.09 ...	38·4°	39·8°	40·3°	39·5°	39·9°	39·3°	39·3°	
	4.12.09 ...	38·3°	39·9°	40·6°	39·7°	40°	40·7°	39·4°	

Slaughtered January, 1910; few paratubercle bacilli; slaughtered because of its giving too little milk.

No. 355 ...	6.4.09 ...	38·7°	40·7°	40·7°	39·9°	40·1°	40·2°	40·1°	

Slaughtered October, 1909; many paratubercle bacilli; slaughtered because of its giving too little milk.

No. 362 ...	6.4.09 ...	38·1°	39·6°	39·6°	39·9°	40·4°	40·5°	40°	
	4.12.09 ...	38·3°	38·7°	39·2°	39·2°	40·3°	40°	39·3°	
	12.4.10 ...	38·6°	38·8°	39·3°	39·2°	39°	39·6°	40·7°	40·5°
	21.11.10 ...	38·7°	38·9°	38·9°	38·9°	38·5°	39·1°	40·2°	39·5°
	31.3.11 ...	38·5°	39·4°	39·1°	38·8°	38·8°	38·7°	39·8°	40·1°

Died of paratuberculosis November, 1911; few paratubercle bacilli.

No. 364 ...	6.4.09 ...	38·6°	40·6°	41·2°	40°	40·3°	40·4°	39·6°	

Slaughtered May, 1909; many paratubercle bacilli; slaughtered because of its giving too little milk.

No. 366 ...	6.4.09 ...	38·3°	38·7°	39°	38·8°	39·5°	39·6°	38·3°	
	4.12.09 ...	38·2°	39·1°	39·4°	39·1°	40·1°	40·1°	39·8°	
	12.4.10 ...	38·4°	39°	38·8°	39·2°	38·3°	40°	39·6°	38·8°

Slaughtered June, 1910; many paratubercle bacilli.

No. 370 ...	6.4.09 ...	38·5°	38·9°	39·2°	38·7°	40·2°	39·5°	38·9°	
	4.12.09 ...	38·3°	38·8°	39°	39°	38·5°	39°	39°	39·7°
	12.4.10 ...	38·3°	38·7°	39·1°	39°	38·6°	38·8°	38·8°	39·2°
	21.11.10 ...	39·7°	39·6°	40°	39·7°	38·7°	39·1°	39·3°	
	31.3.11 ...	38·3°	38·8°	39°	38°	38·3°	38·8°	38·8°	38·5°
	21.11.11 ...	38·2°	38·5°	38·5°	38·6°	38·2°	38·5°	38°	
	16.4.12 ...	38°	38·7°	38·9°	38·5°	38·5°	38·4°	38·2°	

January, 1913.—Intradermic test, 6, 8, 7; complement-fixation test negative. Is still living.

No. 371 ...	6.4.09 ...	38·8°	39·1°	39·5°	38·8°	39·1°	39°	39·1°
	4.12.09 ...	38·4°	38·6°	38·8°	38·8°	38·6°	38·6°	38·6°
	21.11.10 ...	38·5°	38·5°	38·8°	39°	38·8°	38·6°	38·8°
	31.3.11 ...	38·4°	38·9°	38·9°	39·1°	38·8°	38·8°	39°
	21.11.11 ...	39·8°	40·2°	40·4°	40·4°	40·6°	39·4°	40·1°
	16.4.12 ...	38·5°	38·8°	38°	38·4°	38·8°	38°	38·8°

July 12.—Intradermic test, 5, 12, 10, 14; complement fixation test positive. January, 1913.—Complement-fixation test positive; slaughtered August, 1913; few paratubercle bacilli.

		Temperatures before inoculation at 8 p.m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 374	6.4.09	38·1°	39·1°	39·9°	39·6°	40·6°	39·7°	39·1°	
	4.12.09	38·9°	39·5°	40°	40·5°	40·4°	39·9°	39·5°	
	12.4.10	38·3°	38·7°	39·6°	39·3°	38·4°	39·3°	38·7°	
	21.11.10	38·5°	38·8°	39·9°	40·4°	39·5°	39·9°	39°	
	31.3.11	38·3°	38·9°	39·4°	37·5°	38·5°	38·8°	39°	38·4°
	21.11.11	37·7°	38·4°	38·7°	39·2°	39·1°	38·8°	38·3°	
	16.4.12	38·6°	38·5°	37·5°	38·2°	38·9°	38°	38·5°	

July, 1912.—Intradermic test, 5, 12, 12, 14; complement-fixation test negative. Is still living.

No. 388	6.4.09	38·2°	39·7°	40·2°	39·4°	40·4°	39·6°	39·2°	
	4.12.09	38·9°	38·8°	39·7°	39·9°	40·6°	40·8°	40·2°	
	12.4.10	38·5°	39·2°	40·5°	41·2°	39·7°	39·6°	39·6°	

Slaughtered November, 1910; many paratubercle bacilli; slaughtered because of its giving too little milk.

No. 393	6.4.09	38·3°	39·9°	40·4°	39·9°	40·3°	39·5°	39·3°	
	4.12.09	38·8°	39°	39·5°	39·2°	39·8°	39·3°	38·9°	
	12.4.10	38·2°	38·8°	39·1°	39·1°	38·5°	38·7°	38·9°	
	21.11.10	38·8°	39·4°	39·7°	39·7°	39·3°	39·4°	39·3°	
	31.3.11	38·5°	39·1°	39·2°	38·9°	38·7°	38·2°	38·5°	38·4°
	21.11.11	38·2°	38·6°	38·6°	38·9°	38·9°	38·5°	38·2°	
	16.4.12	38·5°	38·8°	39·2°	39·1°	38·5°	38·7°	38·4°	

January, 1913.—Intradermic test, 5, 9, 11, 12; complement-fixation test positive. Is still living.

No. 395	6.4.09	38·6°	39·2°	39·4°	38·7°	40·1°	40·3°	39·3°	
	12.4.10	38·6°	38·6°	39·4°	39·4°	38·9°	39·3°	39·2°	

Slaughtered January, 1911; many paratubercle bacilli.

No. 399	6.4.09	38·3°	39°	39·3°	39·5°	39·7°	38·9°	38·6°	
	4.12.09	38·5°	38·4°	38·9°	38·6°	38·7°	39·5°	38·5°	
	12.4.10	38·3°	38·9°	38·8°	39°	38·6°	38·3°	38·8°	38·9°
	21.11.10	38·5°	38·7°	39·1°	39·2°	39·1°	39·5°	38·9°	
	31.3.11	38·5°	39°	39°	38·8°	38·4°	38·7°	38·8°	
	21.11.11	40·3°	40°	38·7°	38·4°	37·8°	38·2°	38·1°	
	16.4.12	38·4°	38·6°	38·5°	38·9°	38·3°	38·5°	38·3°	

January, 1913.—Intradermic test, 7, 10, 13, 15. Is still living.

No. 402	6.4.09	38·3°	39·7°	40·3°	40°	40·1°	40·4°	39·5°	
	4.12.09	38·4°	39°	40°	40·2°	39·7°	39·6°	39·4°	
	12.4.10	39·2°	39·7°	39·9°	40·4°	40·1°	40°	40·6°	39·6°

Slaughtered November, 1910; few paratubercle bacilli; slaughtered because of its giving too little milk.

No. 412	6.4.09	38·7°	40°	40·3°	39·6°	41·1°	40·6°	40·4°	
	4.12.09	38·3°	38·2°	39·5°	39·9°	40·3°	40·1°	39·4°	

Slaughtered April, 1910; few paratubercle bacilli; no diarrhœa; slaughtered because not pregnant.

		Temperatures before inoculation at 8 p.m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 419 ...	6.4.09 ...	38·4°	39·1°	39·5°	38·4°	38·9°	39·1°	38·7°	
	4.12.09 ...	38·7°	38·9°	39·6°	40°	40°	39·6°	39°	
	12.4.10 ...	38·9°	39·1°	39·5°	39·4°	39°	38·6°	39·2°	38·4°
	21.11.10 ...	38·4°	38·9°	39·6°	39·8°	39·5°	39·8°	39·5°	
	31.3.11 ...	38·5°	38·6°	39·3°	38·6°	38·9°	39·2°	39·1°	38·6°
	21.11.11 ...	38·8°	39·4°	39·8°	40·2°	40·7°	39·7°	39·6°	
	16.4.12 ...	38·5°	38·9°	37·8°	38·3°	38·5°	38·6°	38·5°	

July 12.—Complement-fixation test negative; slaughtered October, 1913; few paratubercle bacilli. The cow was fat.

No. 424 ...	6.4.09 ...	38°	38·9°	39·4°	39·8°	40°	39·9°	38·9°	
	4.12.09 ...	38·4°	38·3°	38·7°	38·6°	38·5°	38·8°	38·7°	
	12.4.10 ...	38·2°	38·5°	38·1°	39·1°	38·3°	39°	38·7°	38·5°
	21.11.10 ...	38°	38·7°	39·2°	39·4°	38·5°	38·6°	39·2°	
	31.3.11 ...	38·5°	38·8°	39°	38·4°	38·7°	38·2°	38·5°	38·4°
	21.11.11 ...	39°	39·1°	39·4°	39·4°	39·4°	39·3°	39°	

January, 1913.—Intradermic test, 5, 6, 6; complement-fixation test positive; is still living.

No. 426 ...	6.4.09 ...	38·2°	40°	40·5°	39·8°	40°	38·8°	38·6°	
	4.12.09 ...	39·4°	39·3°	40·1°	40·3°	40·9°	40·1°	40·2°	
	12.4.10 ...	38°	38·8°	39·2°	39·1°	39°	39·1°	38·2°	38·4°
	21.11.10 ...	39°	38·8°	39·7°	39·9°	39·4°	40·6°	39·7°	
	31.3.11 ...	38·4°	38·3°	38·5°	38·1°	38·8°	38·8°	38·6°	38·8°
	21.11.11 ...	38·7°	38·2°	38·8°	39·2°	38·8°	39·1°	38·5°	
	6.4.12 ...	38·5°	38·9°	38·6°	39°	38·6°	38·6°	38·6°	

Slaughtered June, 1912; many paratubercle bacilli; no diarrhœa; slaughtered because not pregnant.

No. 428 ...	6.4.09 ...	38·6°	39·4°	40·6°	41·4°	40·8°	40·2°	40°	
	4.12.09 ...	38·7°	39°	38·9°	38·8°	38·9°	39°	38·9°	
	12.4.10 ...	38·1°	38·8°	39·2°	38·7°	38·7°	38·8°	39°	38·6°
	21.11.10 ...	38·5°	38·6°	38·8°	38·8°	38·6°	38·6°	39°	
	31.3.11 ...	38·5°	38·5°	38·9°	38·1°	38·3°	38·4°	38·5°	38·6°
	21.11.11 ...	39·3°	39·2°	38·9°	38·8°	39·2°	38·7°	39·1°	
	16.4.12 ...	38·1°	38·4°	38·8°	37·2°	37·8°	38·2°	37·5°	

January, 1913.—Intradermic test, 6, 10, 12, 13; complement-fixation test negative. Is still living.

No. 429 ...	6.4.09 ...	38·8°	40°	41·1°	40·7°	40·9°	39·5°	39·1°

Slaughtered October, 1909; paratuberculosis; had diarrhœa.

No. 430 ...	6.4.09 ...	39·3°	39·5°	39·8°	39·3°	39·8°	40·3°	40·2°	
	4.12.09 ...	38·5°	38·8°	39·1°	38·9°	39·2°	39·2°	39·5°	39·3°

Slaughtered March, 1910; many paratubercle bacilli; no diarrhœa.

		Temperatures before inoculation at 8 p.m. C.	9 hours later C.	11 hours later C.	13 hours later C.	15 hours later C.	17 hours later C.	19 hours later C.	
No. 431 ...	6.4.09 ...	39·4°	39·7°	40·6°	39·5°	40°	40·2°	39·8°	
	4.12.09 ...	39·6°	39·1°	40·5°	40·6°	40·4°	40·5°	40·5°	
	12.4.10 ...	38·5°	39°	39°	39·3°	38°	38·7°	38·8°	39·1°
	21.11.10 ...	39·3°	39·5°	39·5°	39·7°	39·6°	39·4°	39·6°	
	31.3.11 ...	38·5°	39·6°	40·9°	38·6°	39·5°	39·4°	39·4°	39·2°

Slaughtered September, 1911; many paratubercle bacilli.

On the basis of these results I feel warranted in affirming that, *in infected stocks, many animals are suffering from paratuberculosis without the appearance at any time of clinical symptoms of the disease.* The case is thus analogous to what we meet with in the case of tuberculosis in which a very large number of animals are only slightly affected. In conformity with this we find not unfrequently that in such apparently healthy animals only slight intestinal alterations occur and only a few paratubercle bacilli. I have indeed seen cases in which the presence of a few giant cells containing a single paratubercle bacillus could be demonstrated only by means of intestinal sections. On the other side it should be pointed out that it is a frequent occurrence with reacting animals that look the very picture of health to find, when they are killed, their intestines rather extensively thickened and containing a good many paratubercle bacilli.

As evident from the figures quoted *the animals little by little cease to react.* Some stop reacting on the second test already, often reacting again, however, on a later test. Little by little they all cease to react. Thus there was not a single one that reacted to the test on April 16, 1912, most of them reacting again, however, on a later intradermic test with avian tuberculin. So it is, no doubt, quite exceptional that the phenomenon is due to the recovery of the animals. It should be looked upon as a phenomenon of producing immunity. The same fact is well known to occur, although less marked, when tubercular animals are repeatedly injected with tuberculin. The facility with which immunity is produced in animals by means of repeated injections of avian tuberculin is, most likely, connected with the relatively large doses—1·5 c.c. to 2 c.c. of concentrated avian tuberculin—applied twice every year. On the yearly tuberculin test they were further injected with ½ c.c. of concentrated tuberculin, an operation which may not have been quite without influence in that direction.

From the same stock the present writer further received sixty-two intestinal pieces from cows that failed to react to the avian tuberculin tests. In five cases, however, paratubercle bacilli were microscopically demonstrated. In several of these cows, however, some rise of temperature took place on the avian tuberculin test, thus from 38° to 39° C., and from 38·5° to 39·3° C. The sera of all these cows fixed the complement on the complement-fixation test. From cows of the same stock reacting on being tested after April, 1909, intestinal pieces of twenty animals were sent in. Paratuberculosis was shown to exist in sixteen of these cases.

As diagnostic methods for paratuberculosis the *ophthalmic test*, the *cutaneous* and *intradermic tests* with avian tuberculin, have been used too. Horne was the first to give information about the positive ophthalmic test and the cutaneous test in an experimentally infected calf. M'Fadyean, Sheather and Edwards have applied the ophthalmic test in five cases of paratuberculosis, in three cases with a positive result. Of four animals submitted to the intradermic test one only showed a slight reaction. I have myself made a few experiments with ophthalmic tests, but the results were negative.

The *intradermic test seems to be very much inferior to the subcutaneous method*, the rule being, according to my own observations, that severely affected animals fail to react. In this connection it is worth noticing that such animals may react with an intense rise of temperature without showing any local reaction. Thus, I have observed in two cases of tuberculosis-free cows with strongly marked symptoms of paratuberculosis a rise of temperature from 38·3° to 41·5° C., and from 38·5° to 41° C. respectively, after intradermic injection of $\frac{1}{10}$ c.c. of concentrated avian tuberculin, whilst no local reaction appeared. This seems to indicate that avian tuberculin, contrarily to Twort's opinion, is really a rather specific means giving reactions in such small quantities. In order to obtain a rise of temperature on intradermic tests the condition is that the animals have not previously been subcutaneously injected with large doses of avian tuberculin. Thus, of thirty-six formerly reacting animals of a tuberculosis-free stock, one only reacted through rise of temperature after intradermic injection of $\frac{1}{10}$ c.c. of avian tuberculin (39·1° to 40·3° C.), whilst six showed some rise of temperature. If we count a difference of 4 mm. in the thickness of the skin before and seventy-two hours after the injection eighteen of the said thirty-six animals reacted on the intradermic test, the difference with them being 6-7-7-8-4-7·5-4·5-5-8-7·5-6-7·5-6-4-6-6-14 and 9 mm. As will be seen the reactions are considerably slighter than those obtainable in tubercular animals by means of the intradermic method with tuberculin. As far as this stock is concerned, no *post-mortem* examinations are reported. I am going to give some figures from a stock, the results of which were controlled by means of a piece of intestine and some mesenteric glands sent in. In nineteen animals reacting on an intradermic test in January, 1913, a microscopic examination showed eleven cases of paratuberculosis, whereas no such was demonstrated in eight cases. It should be pointed out, however, that five of these eight cases had previously reacted on subcutaneous inoculation. The reactions were as follows (the first figure indicates the thickness of the skin in millimetres before injection, the second, third and fourth figures the thickness of the skin twenty-four, forty-eight, and seventy-two hours later respectively):—

Cow	186	6	10	9	12	paratuberculosis demonstrated.
,,	272	6	13	14	15	,, ,,
,,	342	5	12	12	14	,, ,,
,,	365	6	8	9	11	,, ,,
,,	371	5	12	12	14	,, ,,
,,	380	7	12	14	14	,, ,,
,,	457	7	10	11	10	,, ,,
,,	427	5	12	13	12	,, ,,
,,	521	8	10	12	11	,, ,,
,,	380	7	12	14	14	,, ,,
,,	66	7	—	13	13	,, ,,
,,	281	6	12	13	15	no paratuberculosis demonstrated.
,,	295	6	6	10	11	,, ,,
,,	304	5	10	14	16	,, ,,
,,	408	5	10	12·5	14	,, ,,
,,	487	5	10	11	12	,, ,,
,,	475	6	8·5	8·5	10	,, ,,
,,	496	7	8	10	11	,, ,,
,,	338	5	12	12	13	,, ,,

(338 showed symptoms of the disease.)

In nine cases the animals failed to react to intradermic tests, and yet on microscopic examination numerous paratubercle bacilli were found in the intestines of three animals. In the other six cases no paratubercle bacilli were shown. Measurements taken twenty-four and forty-eight hours only after injection.

Cow	445	7	8	8	no paratuberculosis.
,,	439	7	9	9	,, ,,
,,	42	5	5	5	,, ,,
,,	481	6	8	8	,, ,,
,,	483	5	6	7	,, ,,
,,	452	6	7	7	,, ,,
,,	334	5	8	7	many paratubercle bacilli.
,,	494	5	8	7	,, ,,
,,	497	6	7	7	,, ,,

Thus, a negative reaction in an infected stock does not seem to prove much, as no less than one-third of the animals on *post-*

mortem examination turned out to be affected, although none of them, at the time of the tests, showed any clinical symptom whatever of paratuberculosis. As to the positive reactions, the result was rather misleading too. In fact, as already stated, only in eleven cases of nineteen did the *post-mortem* examination show the presence of paratuberculosis. The positive reactions, however, even if they do not seem necessarily to indicate that the animals are infected, are, in all probability, dependent on the fact that the animals have been exposed to infection. Thus, in a stock in which paratuberculosis was extant one-third of the adult animals reacted to the intradermic test, whereas of forty-eight heifers placed in an isolated stable four only reacted (one of which was afterwards removed owing to paratuberculosis). Twenty-five per cent. of these heifers did not (forty-eight and seventy-two hours after injection) show the slightest thickening of the skin in the place of inoculation, whilst for the rest of them the difference of the thickness of the skin—the reacting animals excepted—in no case exceeded 2 mm.

Animals suffering from paratuberculosis do not react to subcutaneous injection of tuberculin produced from tubercle bacilli of mammals. This clearly results from the fact that in tuberculosis-free stocks in which as many as up to 40 per cent. reacted to the avian tuberculin test, not a single animal reacted to the tuberculin test in which 0·5 c.c. of concentrated tuberculin was applied to adult animals. Nor did cows affected with paratuberculosis, when tested with a larger dose of 2 c.c. of concentrated tuberculin, show any rise of temperature in the experiments I have made; nor did they react to intravenous injection of common tuberculin.

Of tuberculins prepared from other acid-fast bacilli I have experimented with tuberculin prepared from timothy-grass bacilli and from tubercle bacilli of cold-blooded animals, but the results were absolutely negative. As far as the timothy-grass bacilli are concerned Twort and Ingram arrived at the same result.

Agglutination and Complement-fixation Tests.—Twort was the first to publish communications about agglutination and complement-fixation tests in the case of paratuberculosis. In these tests he used emulsions of paratubercle bacilli. The sera of seven naturally infected cattle agglutinated in dilutions of 1 in 10 and 1 in 15. Twort, however, points out that the sera of normal cows may, very likely, often agglutinate in very much the same dilutions. Thus, the test is of no practical value. Twort further examined the agglutinating power of the sera of five calves, two sheep and two goats, that were experimentally infected. Five months after infection the sera of the five calves agglutinated in a dilution of 1 in 5, while the sera of the sheep and goats did not agglutinate. The sera of all animals were tested before inoculation and had shown negative results with the agglutination test. Twort points out that "the agglutination reaction is not specific within the acid-fast group of bacilli. The serum of a tubercular animal will agglutinate an emulsion of Johne's bacillus, and *vice versâ;* the dilution of the serum is, however, usually higher with its homologous bacillus." Twort has further performed a number of agglutination tests with sera of rabbits and fowl inoculated with paratubercle bacilli. Of those animals fowls proved to be the best to produce agglutinins.

Personally, I have performed no agglutination tests. Together with C. W. Andersen I have undertaken such tests with tubercular cows, but as we were unable to find any noteworthy difference in the powers of agglutination of normal and tubercular animals, we considered it hopeless to obtain any practically useful results with paratuberculosis.

Twort has also made experiments with the complement-fixation test in the case of paratuberculosis, using paratubercle bacillus as antigen. Of seven bovines affected with spontaneous paratuberculosis the sera of five fixed the complement, one did not, one gave a doubtful reaction. Of the sera of five experimentally infected calves four gave negative reactions, the fifth a slight positive result, although on *post-mortem* examination the four cases proved affected. Twort realizes the fact that the complement-fixation test, like the agglutination test, is specific within the acid-fast group of bacilli only in a very limited degree. The conclusion Twort arrives at from his researches is that probably neither the agglutination nor the complement-fixation tests will prove of much value as practical diagnostic methods in paratuberculosis. This appreciation is no doubt correct as far as the agglutination test is concerned; whereas, according to O. Bang's and C. W. Andersen's[6] researches, the complement-fixation test is a valuable diagnostic means in paratuberculosis. Certainly it must be admitted that it is absolutely not specific, sera of cows fixing the complement with tubercle bacilli no less than with paratubercle bacilli. We even found in our researches that one of our cultures of tubercle bacilli, originating from a spontaneous case of parrot tuberculosis, and behaving like a typical human tubercle bacillus strain was a very much better antigen towards paratuberculosis than the very paratubercle bacillus. Thus of twenty-five sera of cows that had reacted to avian tuberculin tests, 52 per cent. fixed the complement with the above-mentioned tubercle bacillus, 28 per cent. with the paratubercle bacillus, 24 per cent. with an avian tubercle bacillus. It will thus be impossible to decide whether a fixation is due to tuberculosis or to paratuberculosis. If the complement-fixation test is applied to animals that show suspicious symptoms of paratuberculosis this fact will hardly be of great practical importance, a highly tubercular animal—it is specially such animals that react strongly to the complement-fixation test—being just as worthy of being removed from the stock as an animal affected with paratuberculosis. According to our experience the complement-fixation test has the great advantage always, provided the antigen is good, to give positive results with sera of animals showing clinical symptoms, as sera of such animals fix the complement frequently even in doses of 0·01 c.c. of serum, whereas avian tuberculin, and also paratuberculin, just in such cases prove less reliable.

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[6] *Centralblatt f. Bakteriologie,* u.s.w., 69 Bd., 1913.

We have in all examined sera of about one thousand animals from tuberculosis-free stocks in which the paratuberculosis is of frequent occurrence. In the sections of the stocks in which avian tuberculin failed to react, from 8·5 per cent. to 13 per cent. reacted to the complement-fixation test, a fact that fully explains the failure of eradicating the paratuberculosis from these stocks by means of avian tuberculin, whilst between 37 per cent. and 57 per cent. reacted in the reacting sections. As it is not possible to fix the number of animals of the sections which would have reacted to the injection of avian tuberculin at the time the bloods for the complement-fixation test were taken, it will be of interest to examine the bearing in this respect of stocks that never before were injected with avian tuberculin, and in which the bloods were taken directly before the animals were inoculated with avian tuberculin. Here are a few figures from such stocks:—

In a stock of twenty animals eight fixed the complement. These eight showed the following rise of temperature after injection with avian tuberculin: 38·7° to 40·3° C.—38·5° to 39·9° C.—39·1° to 39·9° C.—38·3° to 40·6° C.—38·5° to 39·6° C.—38·6° to 39·2° C.—38·3° to 39·3° C.—38·6 to 38·8° C. The last three I consider non-reacting. Two animals reacted on the avian tuberculin test, but did not fix the complement.

In another stock consisting of twenty-six animals, six bovines reacted to the complement fixation test. All six also reacted to the avian tuberculin test. In the two animals with the slightest reaction the rise of temperature was from 38·8° to 39·8° C. and from 38·8° to 39·7° C. respectively. One animal reacted to the avian tuberculin test, but failed to fix the complement.

In the third stock, comprehending thirty animals, thirteen of them reacted to the avian tuberculin test, nine of which also reacted to the complement-fixation test. Two gave a positive reaction to the latter, failing to react to the avian tuberculin test.

Of twenty-eight animals tested for the first time and reacting to the avian tuberculin test, thus, twenty also reacted to the complement-fixation test; of forty-eight failing to react five gave complement-fixation reactions, a percentage agreeing well with that of the non-reacting sections. We were, moreover, quite clear that all animals reacting to the avian tuberculin test would not react to the complement-fixation test. In fact, on performing the complement-fixation test with *sera of tubercular animals* it was found that of animals affected with *local tuberculosis* only 14 per cent. reacted, of animals affected with extensive tuberculosis about 97 per cent. when the complement was used in excess, which was the case in all our researches with sera of animals affected with tuberculosis.

As for animals showing *clinical symptoms of paratuberculosis,* we have had the opportunity of examining twenty-six from tubercular stocks. They all fixed the complement. In nine cases we had no opportunty of controlling the result by microscopic examination. Of the remaining seventeen cases sixteen revealed the presence of paratubercle bacilli. The seventeenth case (Cow 338) was, no doubt, a case of paratuberculosis, too, the cow having six consecutive times reacted to the avian tuberculin test, and showed typical clinical symptoms.

From the cows that at the time when the bloods were taken did not show any clinical symptoms, but that still fixed the complement, we have received nineteen pieces of bowels from two stocks. *In all nineteen cases the existence of bacilli was demonstrated, frequently in great numbers.* In two cases, however, it was not till after sections of the intestine had been performed that it was proved to a certainty that the animals had really been affected. We have further received one intestinal piece from one cow showing a doubtful reaction on the complement fixation test. In this case no paratubercle bacilli were demonstrated. In two cases where the animals had shown negative reactions paratubercle bacilli were found when the animals were killed four and eight months respectively after the performance of the test. As to the last of these cases, we were informed that the cow was very fat and had never shown symptoms of paratuberculosis.

On the basis of these researches I feel entitled to maintain *that the complement-fixation test is of no small importance to the diagnosis of paratuberculosis.*

As to the question *whether tubercular cattle would react to the injection of avian tuberculin,* it *a priori* stood to reason that animals infected with avian tuberculosis would react to avian tuberculin. I have myself in three cases inoculated calves that were subcutaneously infected with avian tubercle bacilli first with ordinary tuberculin, then with avian tuberculin. The results were as follows:—

The first figure indicates temperatures before inoculation, the others temperatures nine to twenty-one hours after inoculation.

		C.	C.	C.	C.	C.	C.	C.
(1)	6.10.09 ...	38·2°	38·8°	38·9°	39°	38·9°	39·2°	38·5°
	Inoculated subcutaneously (0·3 c.c. of concentrated tuberculin).							
	18.10.09 ...	38·8°	40·9°	41·5°	41·3°	41·2°	40·9°	41·1°
	Inoculated subcutaneously (1 c.c. of concentrated avian tuberculin).							
(2)	7.9.10 ...	39·1°	39°	39°	39·1°	39·3°	39°	39°
	Inoculated subcutaneously (0·4 c.c. of concentrated tuberculin).							
	9.9.10 ...	39·2°	40·2°	40·1°	40°	40·8°	40·6°	40·8°
	Inoculated subcutaneously (0·4 c.c. of concentrated avian tuberculin).							
(3)	7.9.10 ...	38·5°	39·2°	39·1°	38·8°	39·5°	39·5	39·5°
	Inoculated subcutaneously (0·4 c.c. of concentrated tuberculin).							
	9.9.10 ...	38·9°	40·2°	40·5°	40·5°	40·8°	40·3°	39·7°
	Inoculated subcutaneously (0.4 c.c. of concentrated avian tuberculin).							

As will be seen, all calves reacted typically to injection of avian tuberculin; on the ordinary tuberculin test (1) and (3) showed a rise of temperature from 38·2° to 39·2° C., and from 38·5° to 39·5° C. respectively.

The Royal Commission on Tuberculosis inoculated with avian tuberculin six calves infected with avian tubercle bacilli. Four of them reacted. Of thirteen calves that were infected in the same way one only reacted to an ordinary tuberculin test. It is of greater practical interest to examine *what happens to cattle infected with mammalian tubercle bacilli on being injected with avian tuberculin.* The Royal Commission found that of seven calves infected with mammalian bacilli one only (roughly 11 per cent.?) did not react. I have myself performed the avian tuberculin test on one hundred and six cows in the Copenhagen cattle market.[7] When they were killed fifty-two proved affected by tuberculosis. Of these six gave typical reactions, seven showed some rise of temperature. The following were the temperatures of the thirteen cows previously to and the highest temperatures after inoculation:—

38·7° C.	41·1° C.	38·5° C.	39·5° C.
38·6° C.	40·5° C.	39·1° C.	39·8° C.
38·8° C.	41·4° C.	38·5° C.	39·6° C.
38·8° C.	40° C.	38·4° C.	39·4° C.
38·6° C.	40·8° C.	38·9° C.	39·9° C.
39·6° C.	40·6° C.	38·6° C.	39·5° C.
38·8° C.	39·5° C.		

Even if all the reactions were marked as positive, still 75 per cent. of the tubercular cows would have failed to react, a result which is very different from that of the Royal Commission. Thinking it possible that some of my reactions were due to the tuberculosis of the cows being caused by avian tubercle bacilli, I have in four of the typically reacting cases made cultures and performed inoculation tests on fowls and guinea-pigs. In all four cases bacilli of bovine type were found. Unfortunately, I have had no opportunity of examining the intestines of the cows. Thus it cannot be decided whether some of them being affected with paratuberculosis did react from that reason.

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[7] The animals were subcutaneously inoculated with 2 c.c. of concentrated avian tuberculin in a solution of carbolic acid.

Of the fifty-four bovines that were not tubercular two reacted, most likely owing to paratuberculosis.

On intradermic tests with avian tuberculin ($\frac{1}{10}$c.c. concentrated avian tuberculin) of seventy-three cows that proved tubercular on being slaughtered, twenty-one head reacted (that is, the difference of the thickness of skin before and sixty-five hours after inoculation was, in these cows, between 4 mm. and 12 mm.). On an intradermic test performed simultaneously on the other side of the neck with ordinary tuberculin seventy animals reacted (difference in thickness of skin between 5 mm. and 34 mm.).

Of seventy-four cows that, on being slaughtered, proved to be tuberculosis-free, three reacted to intradermic tests with avian tuberculin. Two of these failed to give a reaction to ordinary tuberculin, whereas the third reacted strongly to the injection of ordinary tuberculin. The intestines were not examined. It is thus difficult to decide whether the cows that reacted to avian tuberculin only were really affected by paratuberculosis. As far as the tubercular cows were concerned, paratuberculosis was hardly of any great importance as a cause of reactions to avian tuberculin, as there is no reason to think that a greater number of the latter than of the tuberculosis-free animals were affected with paratuberculosis (twenty-one of seventy-three as against three of seventy-four).

SUMMARY.

Besides in cattle, spontaneous cases of paratuberculosis have been found in deer, sheep, goat and horse. Experimentally the disease has been transmitted to cattle, sheep, goat and, in two cases, to rabbits. Twort and Ingram have succeeded in preparing pure cultures of the paratubercle bacillus indicating, by adding acid-fast bacilli or extracts of same to the medium, a method by means of which this bacillus may be cultured without difficulty. Twort and Ingram have, from cultures of paratubercle bacilli, produced a paratuberculin, proving the same to be a useful diagnostic means. Twort and Ingram's paratuberculin contains paratubercle bacilli, a state of things which the present writer takes exception to. Cattle affected with paratuberculosis fail to react to ordinary tuberculin. Avian tuberculin may—by preference subcutaneously, less satisfactorily intradermically—be applied as a diagnostic means in paratuberculosis, and has rendered valuable service by giving information about the extent of the disease within affected stocks. Tests performed with avian tuberculin on more than six thousand animals have shown that up to 45 per cent. of adult animals of affected stocks react, while young cattle are affected to a much smaller extent, or, sometimes, are quite healthy. This seems to show that the greater number of young cattle are not infected till the time when they are made to live together with the adults in stables or on pastures. A few animals, however, were infected before they, as new-born calves, were removed from the cowshed. This proves to be the case especially with animals whose mothers are affected with paratuberculosis. I have had the opportunity of following, within a rather large stock, the fates of the cows that reacted to paratuberculin, receiving for examination intestinal pieces, when the cows were removed from the stock. These examinations showed that many of the reacting animals never came to show symptoms of the disease. In other words, paratuberculosis, both in respect of the extent of it within the stocks and in respect of its course, comports itself to tuberculosis. The avian tuberculin test proves less reliable in the case of strongly affected animals, which, as a rule, however, will react. But the means did not prove absolutely reliable either when tests were performed on apparently healthy animals. This is the reason why it has not as yet been possible to clear out affected stocks by isolating the reacting animals (Bang's system). It is to be hoped that paratuberculin will become a means of solving this problem.

The complement-fixation test has proved a valuable diagnostic means in paratuberculosis in the case of tuberculosis-free stocks. All severely affected animals give positive reactions on this test.

Tubercular animals will sometimes react to the avian tuberculin test. According to my researches 21 per cent. of tubercular animals reacted to subcutaneous avian tests, if a rise of temperature of 1° or above is counted a positive reaction. On intracutaneous tests about 29 per cent. reacted, if a difference in thickness of skin of 4 mm. before and seventy-two hours injection is counted a positive reaction. From the researches published up to now I draw the following conclusions:—

(1) Paratuberculosis is a specific disease caused by the paratubercle bacillus.

(2) In stocks that sustain a loss through the disease a very large number of adult animals is really affected, while the young cattle are either quite healthy, or, anyhow, much less affected.

(3) Many affected animals will never show any symptoms of the disease.

(4) The disease should be fought against by removing affected animals from healthy ones.

Dixième Congrès International de Médecine Vétérinaire, Londres, 1914.

La Paratuberculose.

Résumé du Rapport du M. OLUF BANG.

En dehors des bovidés, des cas spontanés de paratuberculose ont été observés chez le daim, le mouton, la chèvre et le cheval. Expérimentalement la maladie a été transmise aux bovidés, au mouton, à la chèvre et dans deux cas au lapin. Twort et Ingram ont réussi à obtenir des cultures pures du bacille paratuberculeux par addition de bacilles acido-résistants ou de leur extrait au milieu, méthode au moyen de laquelle la culture de ce bacille peut être obtenue sans difficulté. Twort et Ingram ont obtenu des cultures du bacille paratuberculeux une paratuberculine et ont prouvé en même temps qu'elle pouvait être utilisée comme moyen de diagnostic. La présence de bacilles paratuberculeux dans la paratuberculine de Twort et Ingram constitue un état de choses au sujet duquel l'auteur fait des réserves. *Les bovidés atteints de paratuberculose ne réagissent pas à la tuberculine ordinaire. La tuberculine aviaire*—de préférence en injection souscutanée, moins bien en injection intradermique—peut être utilisée comme moyen de diagnostic et *a rendu des services appréciables pour renseigner sur l'extension de la maladie dans les troupeaux affectés.* Les épreuves réalisées avec la tuberculine aviaire sur plus de six mille animaux ont montré que jusqu'à 45 pour cent des animaux adultes réagissent dans les exploitations infectées, tandis que la proportion est plus faible chez les jeunes bovidés et que quelquefois ces derniers sont tout à fait sains. Ceci semble montrer que le plus grand nombre des jeunes bovidés ne sont pas infectés jusqu'au moment où ils sont mis en commun avec les adultes à l'étable ou au pâturage. Quelques animaux étaient déjà atteints comme veaux nouveaux nés avant d'avoir quitté leur mère. J'ai eu l'occasion de suivre dans un grand troupeau le sort des vaches qui réagissaient à la tuberculine aviaire en examinant l'intestin des animaux lorsqu'ils étaient exclus de l'exploitation. Ces examens ont montré *que beaucoup des* *animaux réaggissants n'ont montré aucun signe de la maladie.* En d'autres termes *la paratuberculose envisagée par rapport à son extension dans le troupeau et par rapport à son évolution se comporte d'une manière analogue à la tuberculose.* L'épreuve à la tuberculine aviaire a moins de valeur chez les animaux gravement affectés qui cependant, en règle générale, réagissent. Mais le procédé ne se montre pas non plus d'une valeur absolue quand les épreuves sont réalisées chez des animaux présentant les signes de la santé. Ceci explique pourquoi il n'a pas été possible d'épurer complètement les troupeaux infectés par l'isolement des animaux réagissants (système de Bang). Il est à espérer que la paratuberculine soit le moyen de résoudre le problème.

La réaction de fixation du complément s'est montrée comme un bon moyen de diagnostic de la paratuberculose dans les cas des troupeaux indemnes de tuberculose. Tous les animaux gravement infectés réagissent positivement à cette épreuve.

Les animaux tuberculeux réagissent quelquefois à la tuberculine aviaire. D'après mes recherches 21 pour cent des animaux tuberculeux réagissent à l'épreuve souscutanée pratiquée avec la tuberculine aviaire, si une hyperthermie de 1° ou au-dessus est considérée comme réaction positive. Vingt-neuf pour cent réagissent à l'inoculation intracutanée si une difference de 4 mm. entre l'épaisseur de la peau avant l'inoculation et 72 heures plus tard est considérée comme réaction positive. Des recherches publiées jusqu'à maintenant je tire les conclusions suivantes :—

(1) La paratuberculose est une maladie spécifique déterminée par le bacille paratuberculeux.

(2) Dans des troupeaux où la maladie a causé des pertes sensibles un très grand nombre des animaux adultes est réellement infecté tandis que les jeunes sont ou tout à fait sains ou infectés dans une proportion beaucoup moindre.

(3) Beaucoup d'animaux atteints ne présentent jamais les signes de la maladie.

(4) La maladie doit être combattue par la séparation des animaux atteints des animaux sains.

Zehnter internationaler tierärztlicher Kongress in London, August, 1914.

AUSZUG aus dem BERICHT von Tierarzt OLUF BANG.

AUSSER beim Rinde wurden spontane Fälle von Paratuberkulose beim Hirsch, Schaf, Ziege und Pferd festgestellt. Experimentell wurde die Krankheit übertragen auf Rind, Schaf, Ziege und in zwei Fällen auf Kaninchen. Twort und Ingram ist es gelungen, Reinkulturen des Paratuberkelbazillus herzustellen und zwar durch Hinzufügen säurefester Bazillen oder deren Extrakte zu dem Nährboden, eine Methode, vermittels der dieser Bazillus ohne Schwierigkeiten gezüchtet werden kann. Twort und Ingram haben aus Kulturen des Paratuberkelbazillus ein Paratuberkulin hergestellt, das sich als ein brauchbares Diagnosticum erweisen hat. Twort und Ingrams Paratuberkulin enthält jedoch Paratuberkelbazillen, ein Umstand, den der Berichterstatter nicht gutheissen kann. Mit Paratuberkulose behaftete Rinder reagieren nicht auf gewöhnliches Tuberkulin. Geflügeltuberkulin kann—besonders subkutan, mit weniger Erfolg intrakutan—als Diagnosticum bei Paratuberkulose angewandt werden und hat wertvolle Dienste geleistet, indem es über die Ausdehnung der Seuche innerhalb der befallenen Bestände Aufklärung gab. Impfungen mit Geflügeltuberkulin bei mehr als 6000 Tieren haben gezeigt, dass bis zu 45 pro Cent. der erwachsenen Tiere der verseuchten Bestände reagieren, während die Jungrinder zu einem viel geringeren Prozentsatz verseucht oder auch bisweilen ganz gesund sind. Daraus scheint hervorzugehen, dass die grössere Zahl der Jungrinder nicht infiziert ist bis zu der Zeit, wo sie gezwungen sind, mit den älteren Tieren in Ställen oder auf der Weide zusammen zu leben. Einige Tiere jedoch waren schon als neugeborene Kälber infiziert, bevor sie den Stall verliessen. Dies ist besonders der Fall bei den Tieren, deren Mütter an Paratuberkulose leiden. Ich habe Gelegenheit gehabt, in einem ziemlich grossen Bestande die Schicksale der Kühe, die auf Geflügeltuberkulin reagierten, weiter zu verfolgen, indem ich zur Untersuchung Darmstücke erhielt, als die Kühe aus dem Bestand ausgemerzt wurden. Diese Untersuchungen ergaben, dass viele der reagierenden Tiere überhaupt keine Krankheitserscheinungen aufwiesen. Mit andern Worten: Die Paratuberkulose verhält sich sowohl hinsichtlich ihrer Ausbreitung innerhalb der Viehbestände als auch hinsichtlich ihres Verlaufes ganz ähnlich wie die Tuberkulose. Die Impfung mit Geflügeltuberkulin erweist sich weniger zuverlässig bei schwer erkrankten Tieren, die jedoch meistens reagieren. Das Geflügeltuberkulin erwies sich aber auch dann nicht als absolut zuverlässig, wenn die Impfung an auscheinend gesunden Tieren vorgenommen wurde.

Dies ist der Grund, warum es bisjetzt noch nicht gelungen ist, verseuchte Bestände durch Isolierung der reagierenden Tiere (Bangsches Verfahren) zu säubern. Es ist jedoch zu hoffen, dass wir in dem Paratuberkulin ein Mittel erhalten, dieses Problem zu lösen.

Die Komplement-Ablenkungsmethode hat sich in tuberkulose-freien Beständen als ein wertvolles Diagnosticum für die Paratuberkulose erwiesen. Alle schwer erkrankten Tiere reagierten positiv auf diese Probe.

Tuberkulöse Tiere reagieren zuweilen auf das Geflügeltuberkulin. Nach meinen Untersuchungen reagierten 21 pro Cent. tuberkulöser Tiere auf die subkutane Impfung mit Geflügeltuberkulin, vorausgesetzt, dass eine Temperatursteigerung von 1 Grad oder darüber als eine positive Reaktion angesehen wird. Auf die intrakutane Impfung reagierten etwa 29 pro Cent., vorausgesetzt, dass es als eine positive Reaktion gilt, wenn die Dicke der Haut Zweiundsiebzig Stunden nach der Injektion 4 mm. mehr beträgt als vor der Injektion. Aus den bisher veröffentlichen Untersuchungen ziehe ich folgende Schlùsse :—

(1) Paratuberkulose ist eine spezifische Krankheit, verursacht durch den Paratuberkelbazillus.

(2) In Beständen, die durch diese Krankheit Verluste erleiden, ist eine sehr grosse Zahl erwachsener Tiere wirklich verseucht, während die Jungrinder entweder ganz gesund oder auf jeden Fall viel weniger verseucht sind.

(3) Viele verseuchte Tiere weisen niemals Krankheitserscheinungen auf.

(4) Die Seuche muss durch Trennung der verseuchten Tiere von den gesunden bekämpft werden.

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